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CARDIAC PERFORMANCE IN RESPONSE TO LOADING PRESSURES IN *BUSYCON CANALICULATUM* (GASTROPODA) AND *MERCENARIA MERCENARIA* (BIVALVIA)

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SUMMARY

The performance of the isolated hearts of a gastropod, *Busycon canaliculatum* (L.), and a bivalve, *Mercenaria mercenaria*(L.), were examined at different perfusion levels around the expected physiological ranges. Both hearts followed the Frank-Starling relationship with regard to stroke volume *versus* preload, but the heart-rate response was species-dependent. The argument is developed that the molluscs might functionally apply Starling's Law of the heart to accommodate increased output during exercise.

At the expected *in vivo* filling pressures the power output of the two hearts was the same ($15–30 \times 10^{-6}$ W g^{-1} ventricular tissue), but the *Mercenaria* filling levels were considerably lower. This clearly indicates that the cardiac muscle of each of the two species has evolved to operate at specific pressure ranges.

Electrical recordings from the surface of the myocardium in the perfused *Busycon* hearts confirm that the shape changes reported in the literature, dealing with stretched myocardium, also occur for changes in the whole heart at realistic loading pressures. These results support previous conclusions that the cardiac output is controlled by the duration of the action potential plateau.

INTRODUCTION

This paper examines the heterometric and homeometric autoregulation of the heart of a gastropod and a bivalve. The molluscs are a diverse phylum and such a study is necessary in order to understand the evolutionary changes which ultimately lead to the highly responsive cardiovascular systems of the cephalopod molluscs. Two studies have examined the energetics of the isolated ventricle of the dibranchiate octopods Smith, 1981*a*; L. Foti, I. T. Genoino & C. Agnisola (in preparation), but both studies have the problem that the structure of the octopod ventricle is complex with its own coronary vascular supply and, certainly in the case of the *Eledone* species,

Key words: Heart, energetics, Mollusca.

the possibility that the isolated heart is not denervated (Smith & Boyle, 1983). It is apparent, therefore, that in order to understand the intrinsic functional characteristics of the molluscan myocardium we must turn to the 'lower' molluscs, such as the gastropods and bivalves, where the cardiac structure is simpler.

Most work on isolated perfused molluscan hearts has concentrated on bioassay use, although a few authors have examined molluscan myocardial energetics in more detail. Straub (1901) showed that both pulse frequency and amplitude increased with the internal perfusion pressure and that stroke volume increased proportionally up to a perfusion pressure of 2 cmH_2O, above which output declined (Straub, 1904). However, these studies as well as more recent ones (for example, Schwartzkopff, 1954; Civil & Thompson, 1972; Sommerville, 1973) were not conducted in a way which allows the calculation of work done and power output. Most commonly, the output pressure on the heart was less than the venous input or the absolute levels of perfusion or heart weights are not given. Only Herold (1975) has studied a cannulated preparation where the estimation of power output is possible. His data, when corrected for ventricular weight, give a value for the heart of *Helix* of 103×10^{-6} W g^{-1} ventricular tissue at realistic *in vivo* perfusion pressures. Unfortunately, the *Helix* ventricles in Herold's study were perfused with Straub cannulae which do not differentiate between preload and afterload nor do they hold the afterload constant. Such controls are necessary in order to calculate a realistic power output. No data are available for the bivalve heart.

The two species in this study are the gastropod *Busycon canaliculatum* and the bivalve *Mercenaria mercenaria*. The isolated hearts are examined with the emphasis on their performance in response to changes in perfusion levels. The electrical activity of the myocardium is related to performance by recording with externally applied suction electrodes while measuring cardiac output. This method is a great improvement over the electrocardiogram in providing a faithful representation of the time course of the compound action potential of the myocardium. Irisawa, Kobayashi & Matsubayashi (1961) used the floating microelectrode method to show that the oyster myocardium possesses cellular action potentials of a 'cardiac' type, with a prepotential, spike and a plateau on the repolarization, as well as action potentials which repolarize quickly. These authors also demonstrated that extracellular suction electrode records from whole hearts closely resembled intracellular records, although amplitude was reduced to about one-tenth. Nevertheless, both types of action potentials could be recorded. The suction electrode was recognized as the method of choice for continuous observation of the change in form of action potentials in response to pharmacological treatment which affected the duration of the plateau phase (Irisawa *et al.* 1961). Hill & Irisawa (1967) used the suction electrode method with the ventricle of a large marine gastropod, *Rapana thomasiana*. Simultaneous suction electrode and mechanogram recording showed that the phases of the suction electrode record preceded the phases of the mechanogram, demonstrating that the suction electrode record is not a mechanical artifact. Reduced perfusion pressure led to dedifferentiation of the suction action potential, with loss of the spike and plateau phases and corresponding diminution in force (Hill & Irisawa, 1967). This paper provides evidence from simultaneous suction and pressure recordings for a myocardial response to changes in perfusion pressure which is mediated through changes in the form of the action potential.

MATERIALS AND METHODS

Two species of mollusc were studied, *Busycon canaliculatum* (Gastropoda) and *Mercenaria mercenaria* (Bivalvia). The work was carried out at the Department of

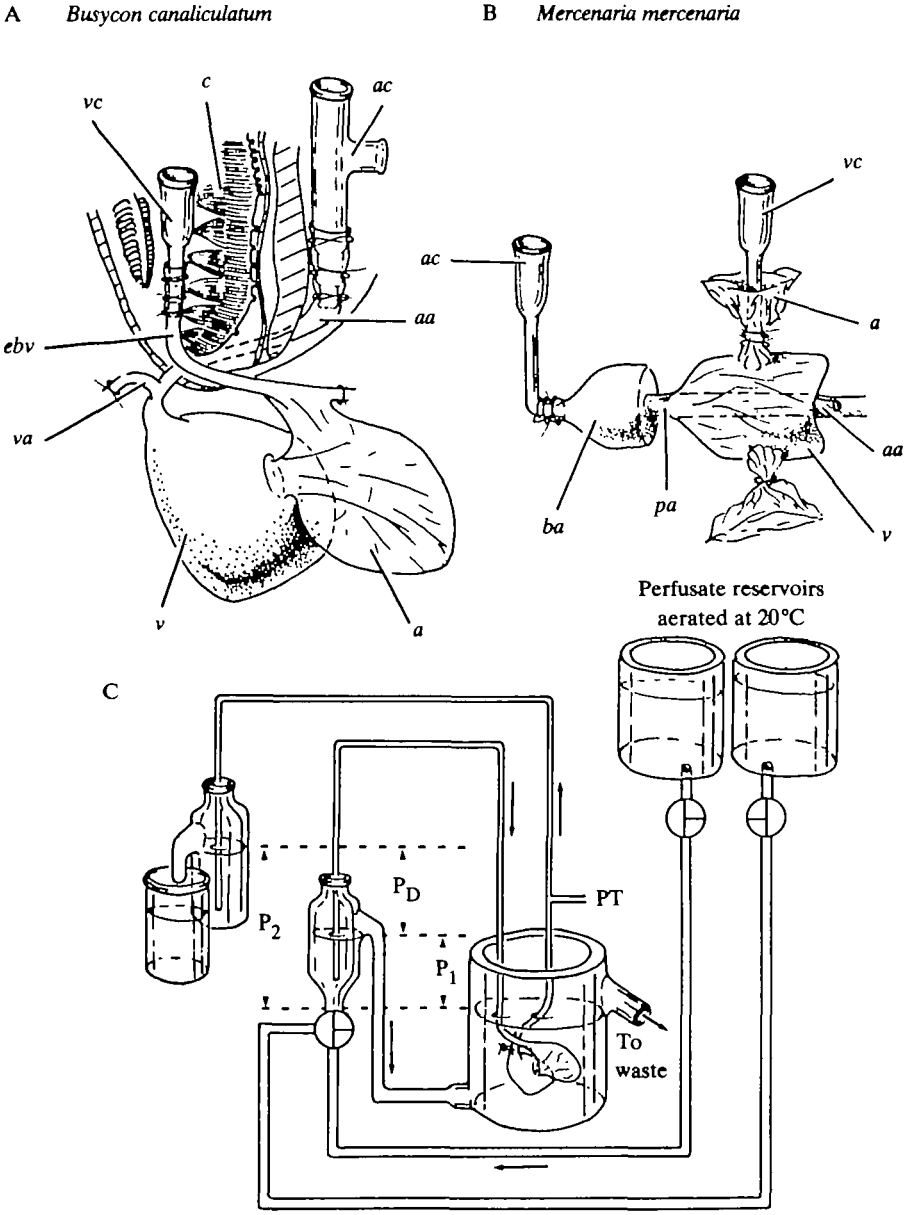

Fig. 1. (A) Semi-diagrammatic representation of the cannulated systemic heart of the gastropod *Busycon canaliculatum*. (B) Semi-diagrammatic representation of the cannulated heart of the bivalve *Mercenaria mercenaria*. *a*, atrium; *aa*, anterior aorta; *ac*, aortic cannula; *ba*, bulbus arteriosus; *c*, ctenidium; *ebv*, efferent branchial vessel; *pa*, posterior aorta; *v*, ventricle; *va*, visceral aorta; *vc*, venous cannula. (C) Diagram of the perfusion system. P_1 is the preload, P_2 is the afterload and P_D is the pressure difference between the two, against which the heart works. PT is the side arm of the aortic cannula to which the pressure transducer is connected.

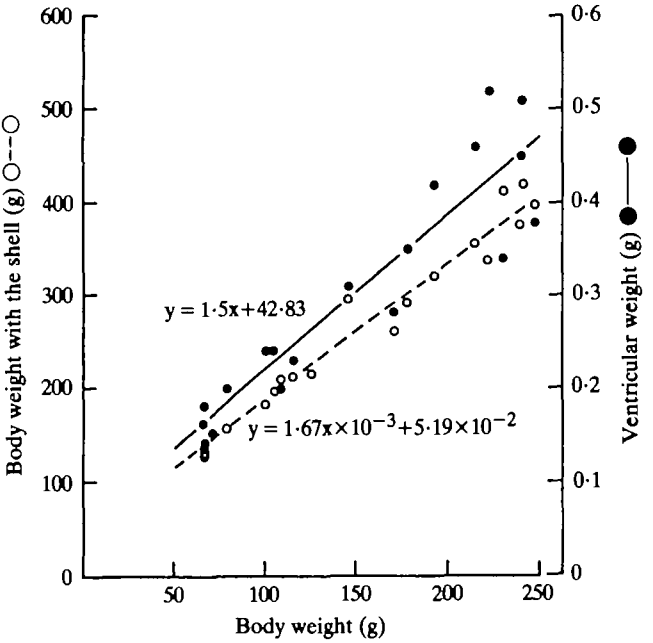

Fig. 2. The relationship between body weight, ventricular weight and the body weight with the shell for *B. canaliculatum*. The regression lines are also plotted.

Zoology, University of Rhode Island, U.S.A. Bivalves were acquired locally from commercial fish markets, as were the smaller *Busycon*. Larger specimens of *B. canaliculatum* came from the Marine Biological Laboratory, Woods Hole, Massachusetts. Both sexes were used.

The essential part of setting up an isolated working heart was to cannulate both the venous return and the aortic output. The cannulae could then be led to perfusion reservoirs providing controlled preloads (P_1 = venous return) and afterloads (P_2 = aortic back pressure). Perfusion pressures were chosen by referring to literature values on *in vivo* pressures. Within the Bivalvia vascular pressures appear relatively consistent with atrial pressures of 0·2–1·5 cmH_2O. Ventricular systolic pressures range from 1·5–6 cmH_2O (Brand, 1972; Florey & Cahill, 1977). Gastropod pressures are more variable and the levels used in this study are for marine prosobranchs. *Haliotus* has an atrial pressure of 1·1–2·2 cmH_2O and an aortic systolic pressure of 8·8 cmH_2O with a diastolic level of 5·9 cmH_2O (Bourne & Redmond, 1977) *Patella* has an atrial pressure of 1–3·5 cmH_2O and an intraventricular systolic pressure of 5 cmH_2O (Jones, 1970).

Busycon were removed from the shell and the foot was pinned to a dissection dish. A cut was made in the efferent branchial vessel at the distal end of the osphradium. A venous input, blunt-ending Luer-fitted cannula was then pushed into the vessel up to the point where it turns a right angle into the atrium. Either of two cannula diameters were used (internal diameters of 0·75 or 1·25 mm) depending on the size of the animals. Shallow Araldite rings along the cannula length were used to lock the sutures in place as they passed through the base of the gill. The afferent branchial vessel was tied off to prevent backflow. To install the aortic cannula the body wall was

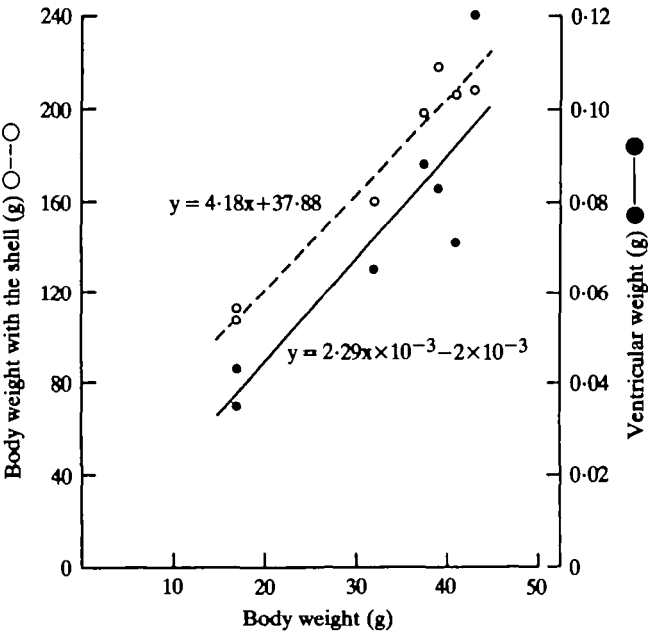

Fig. 3. As in Fig. 2 but for the bivalve *M. mercenaria*.

opened and the major anterior aorta dissected out from the anterior end of the body cavity (for terminologies see Dakin, 1912). A length of the aorta was freed to half-way down the cavity. The anterior end of the vessel was cut and a rabbit tracheal cannula (with the side arm opened) pushed in to the point where the aorta narrows and dives into the body wall. More distal to this point the aortic wall was weaker and its path convoluted. The cannula was tied in place by ligatures passing into the body wall for support. At this stage the heart, in the pericardium, was removed with the cannula attached to pieces of the body wall for support. Venous return from the kidney and the visceral artery were ligatured. The heart was then freed from the pericardium and unnecessary tissues were trimmed off (Fig. 1A). In this condition there should not be neural influence on the heart (Kuwasawa & Hill, 1973). Since this preparation included a working atrium and ventricle, care had to be taken thoughout that neither were punctured. This preparation has also been successfully made with *B. carica* and *B. contrarium*.

The removal of the bivalve heart is simpler than in *Busycon* but the major problem is supporting it in the perfusion apparatus. The following method is a solution to this problem. *M. mercenaria* is first removed from its shell and the anterior aorta ligatured, with the attached thread being used to handle the preparation. The ventricle, both atria and the bulbus arteriosus still attached to the posterior aorta, are freed and lifted out. One atrium and the bulbus are cannulated (i.d. 1·25 mm and 0·75 mm respectively), the other atrium is ligatured (Fig. 1B). The preparation and cannulae are then attached to a sheet of thick plastic by the ligature threads. The ventricle is left free to fill and contract but the weight of the cannulae are supported. *Mercenaria* terminologies are based on those for *Cardium* (Johnstone, 1899).

As the aim of the experiments reported here is to investigate the intrinsic properties

of the cardiac muscle, the hearts have been removed from the pericardium. It seems unlikely that the degree of distension observed during these experiments would occur within the constraints of the pericardium.

In both preparations the perfusion system was similar to that described by Smith (1981*a*) for work on the isolated heart of octopus. Two independent reservoirs (Fig. 1C) fed the input cannula (preload, P_1) and provided a constant output pressure (afterload, P_2). The heights of the reservoirs were measured from the water level in the organ chamber. As perfusion pressures were measured in cmH_2O, this is used on the x-axis of the graphs. In order to calculate work and power levels the pressures are converted to Pascals (1 $cmH_2O = 98{\cdot}1$ Pascal). Fresh filtered sea water at 20 °C fed both reservoirs and the organ bath. Mean stroke volumes were measured by collecting and weighing the overspill from the output reservoir over a known number of heart beats. The weight was converted to a volume, taking into account specific gravity and temperature. Heart rate was measured by recording and counting the number of aortic pressure pulses using a Harvard Instruments pressure transducer installed in the T-piece of the output cannula. The output was amplified using a Grass low level d.c. preamplifier (7P1B). Heart rate is therefore equivalent to the number of contractions strong enough to exceed the diastolic 'aortic' afterload opening the aortic valve and not necessarily to the mechanical or electrical activity of the myocardium. As will be seen in the results the two methods do not always agree.

Myocardial electrical activity was recorded using suction electrodes applied to the outside of the perfused and contracting heart. These electrodes were made using silver-silver chloride wire, and signals were amplified with a Grass low level d.c. preamplifier (7P1B). In *Busycon* the electrode was applied between the atrial/ventricular valve and the ventricular/aortic valve. In the organ chamber this was the uppermost and most stable surface. In *Mercenaria* the electrode was applied to the surface that *in vivo* abuts the shell.

All hard copy, of both pressure and electrical signals, was made on a Grass Polygraph 79C fitted with d.c. driver amplifiers (7Da) and giving a curvilinear output.

After each experiment the ventricle, cleaned of excess tissue, including the atrium, was blotted with absorbent paper to remove surface water and weighed. All the results are therefore expressed in terms of the ventricular wet weight. Body weights were measured when the animals were fully retracted into their shells, or in the case of *Mercenaria* when the shell was opened and the water drained out.

RESULTS

The output of the heart, the stroke work and the power values are expressed per gram wet ventricular tissue. To allow back calculation to the measured stroke volume values, Figs 2 and 3 give the relationship between body weight, with and without the shell, and the ventricular weights for both *Busycon canaliculatum* and *Mercenaria mercenaria*. Not surprisingly, the variables are significantly related and, over the range examined, show linear relationships. In the following experiments on *B. canaliculatum*, animals at the lower end of the size range were used, with heart weights between 0·2 and 0·35 g. Over an extended size range (heart weights from 0·15

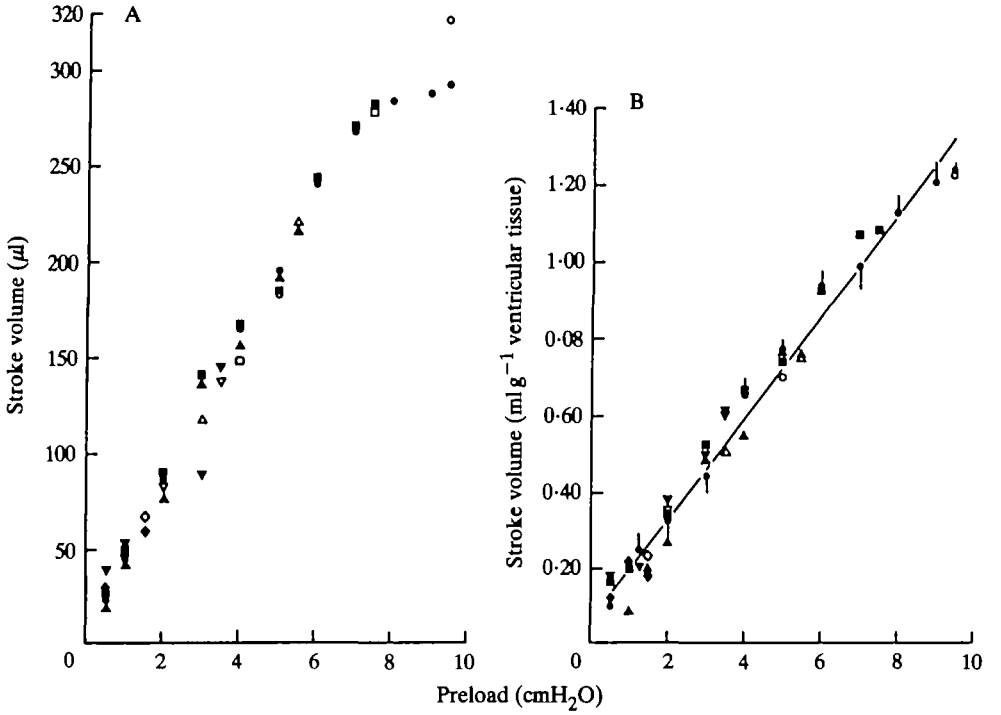

Fig. 4. (A) The effect of changing the preload value on the stroke volume of the *Busycon* heart at different afterloads. The data are from a single preparation with no correction for heart weight. Afterload values are: 10 cmH$_2$O (●), 8 cmH$_2$O (■), 6 cmH$_2$O (▲), 4 cmH$_2$O (▼) and 2 cmH$_2$O (◆). The ventricular weight is 0·23 g. (B) As in A but here the data for all the preparations have been pooled after correction for the different ventricular weights. Single standard errors are shown on either side of the mean values for the preload changes at an afterload of 10 cmH$_2$O. In both Fig. 4A and B and all subsequent figures the open symbols are stroke volume values as the preload is returned to the starting pressure. In no case is there any indication that the heart has deteriorated during the run.

to 0·66 g: P. J. S. Smith, unpublished observation) a doubling of weight correlates with more than twice the stroke volume. The outputs presented in this paper apply, therefore, to the size range used.

Energetics of the Busycon *heart*

On removal from the animal, the systemic heart showed no sign of contractile activity, but when internally stretched by a perfusion head of between 6 and 9 cmH$_2$O, both the ventricle and atrium contracted regularly for over 12 h. The regular activity allowed the performance of the heart to be measured over a series of pre- and afterload values. After installing the heart in the perfusion system, it was allowed to beat for 1 h at constant perfusion conditions (preload 9·5; afterload 10 cmH$_2$O). Following any change in loading, measurements were not made for at least 3 min. Mean stroke volume was then measured over 20–30 contractions. Heart rate was counted over a 2- to 3-min period during and after stroke volume collection. (This procedure was also followed for *Mercenaria*.) Of ten preparations examined at five preset afterloads, five were clearly leaking at the lower pressure levels and have not been included in the following results. Leaking could be clearly seen by a drop in the

afterload pressure level during the diastolic period. A further three preparations were examined at an afterload of 8 cmH_2O.

The stroke volume from the isolated heart ranged in proportion to the size of the heart. During this study, outputs of between 40–150 μl stroke^{-1} were measured at a preload of 3 cmH_2O and an afterload of 6 cmH_2O. Fig. 4A gives an example of the measured stroke volume from a single preparation where the afterload had been preset at several different levels and the preload varied by 1-cmH_2O decrements. The results are not corrected for ventricular weight. When corrected, data from different preparations can be pooled (Fig. 4B). In both these figures it is clear that over the experimental pressures used, stroke volume varies in a direct relationship with the preload. Afterload values have no effect on the output. The relationship for the corrected and pooled data is highly significant ($r = 0{\cdot}99$; $P < 0{\cdot}001$) fitting a linear regression equation of:

$$\text{stroke volume (ml)} = 134 \times 10^{-5}\, P_1 + 743 \times 10^{-4},$$

where P_1 is the preload.

Heart rate increased up to a threshold at 4–6 cmH_2O and then remained constant at about 12–13 beats min^{-1}. The data from both the individual preparations (see for an example Fig. 5A) and the pooled results (Fig. 5B) suggest that the relationship is an approximation to an exponential function with the equation:

$$\text{heart rate (beats min}^{-1}) = 13\,(1 - e^{-68 \times 10^{-4} P_1}).$$

The sum of the squares is 23·2 ($N = 37$ heart rate values from all the preparations). The expected *in vivo* atrial pressures would be somewhere between 2 and 6 cmH_2O, implying that the susceptibility of the heart to changes in the venous return pressure is maximal over the expected operating range. The amplitudes of the aortic pressure pulses are unaffected by afterload but ranged from 0·5 cmH_2O at 1·0 cmH_2O to 3 cmH_2O at 9·5 cmH_2O preload.

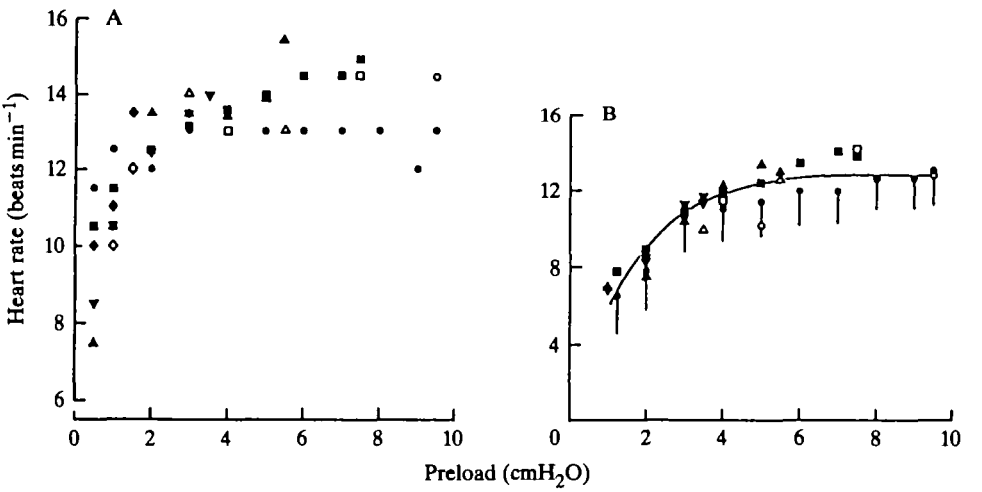

Fig. 5. (A) Contraction rate of the *Busycon* systematic heart from a single preparation in response to changes in the preload level at different set afterloads. (B) As in A but combining the data from all the preparations. Standard errors are shown on one side of the means for the run at an afterload of 10 cmH_2O. Symbols as in Fig. 4.

Electrical records from the myocardial surface, when the heart is perfused at preload levels below 4 cmH_2O, can show apparently normal activity, correlating with visually observed contractions, which are not strong enough to open the aortic valve (Fig. 6A). As heart rate is measured from aortic pressure pulses, the recorded rate is not equivalent to a rate measure based on the electrical records. In some cases a clearly abnormal and rapid electrical signal is recorded (Fig. 6B) without any sign of synchronous mechanical activity.

Using the results of stroke volume and heart rate changes in response to varying the pre- and afterload levels, it is possible to calculate the stroke work and power output of the *Busycon* systemic heart. These are shown in Figs 7 and 8. Power is calculated as:

$$\text{power (Watts)} = \text{stroke work (J)} \times \text{frequency } (s^{-1})$$
$$= \text{stroke volume } (m^3) \times P_D \text{ (Pascals)} \times \text{frequency } (s^{-1}),$$

where P_D is the pressure difference (afterload minus preload). Semi-empirical curves for stroke work at the different afterloads can be calculated from the regression equation of stroke volume against preload. A similar relationship can be arrived at for the power

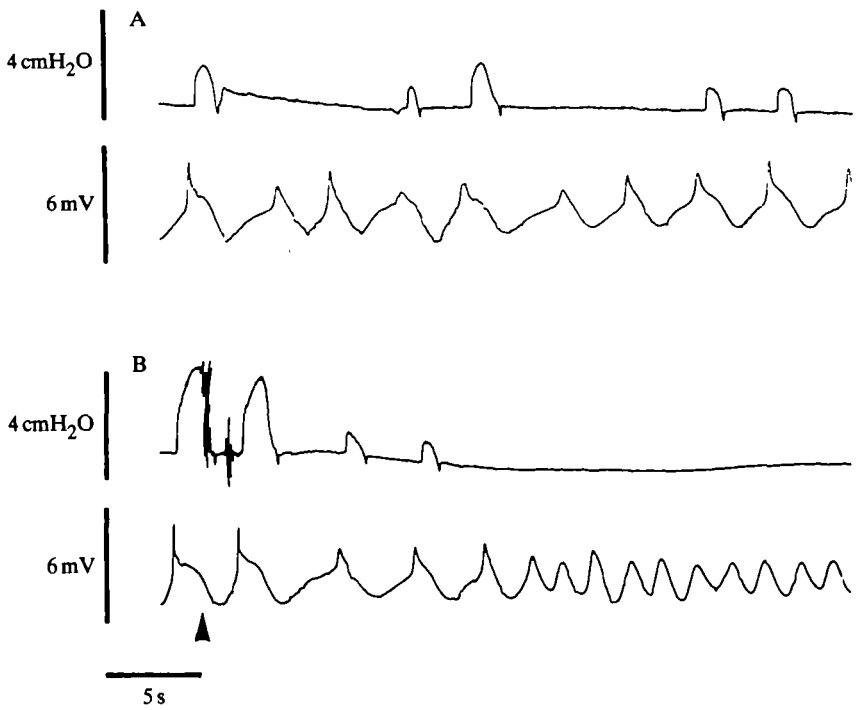

Fig. 6. Unusual electrical activity, which did not correspond to aortic pressure pulses, recorded from the *Busycon* ventricle at low perfusion pressures (preload = 1 cmH_2O; afterload = 10 cmH_2O). (A) Irregular activity (which corresponds to observed muscular contractions) which only intermittently gave rise to 'aortic' pressure pulses. (B) Regular rapid electrical activity in the absence of either visible muscular contractions or 'aortic' pressure pulses. The arrowhead denotes the point where preload was reduced from 9·5 to 1 cmH_2O.

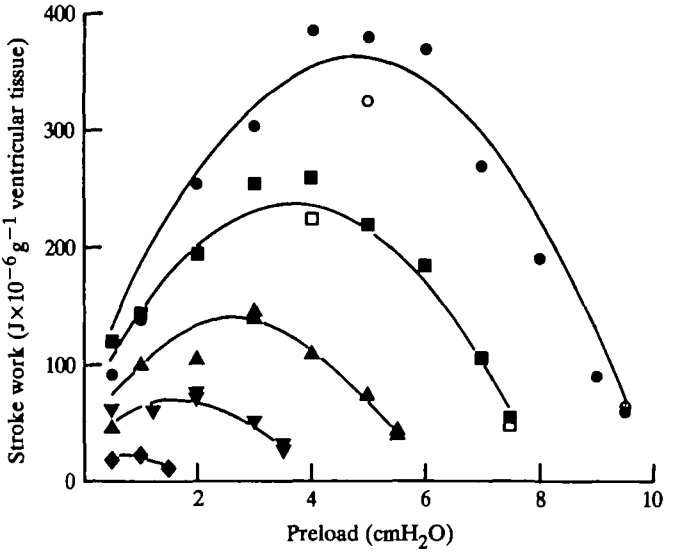

Fig. 7. Stroke work curves for *Busycon* calculated from the pooled data at changing preloads and preset afterloads. The calculated semi-empirical curves, based on the regression equation for Fig. 4B, are fitted over the measured data. Symbols as in Fig. 4.

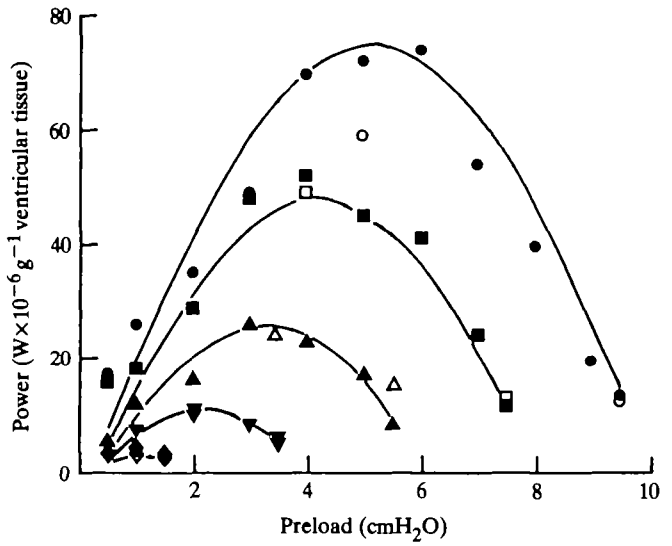

Fig. 8. Power curves calculated from the pooled *Busycon* data. Afterload is preset and the preload is varied. The semi-empirical curves, based on the equations for the stroke volume and heart rate, are fitted over the measured data. Symbols as in Fig. 4.

output by relating stroke work to time. The semi-empirical equation for this is: power $(\times 10^{-6}\,W\,g^{-1}) = [134\times10^{-5}\,P_I\,(P_D) + 743\times10^{-5}\,P_D]\times[217\times10^{-3}\,(1-e^{-68\times10^{-4}\,P_I})]$. The power curves fall off more rapidly below the preload level of 4 cmH_2O as a result of the rapid reduction in heart rate.

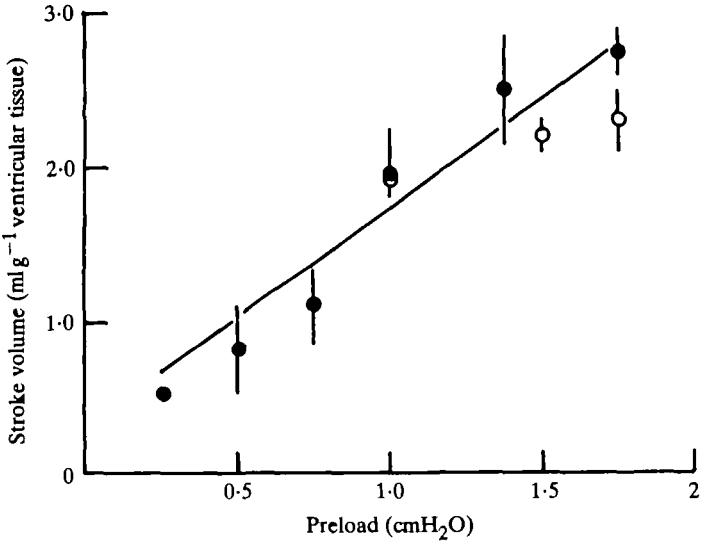

Fig. 9. The effect of changing the preload value on the stroke volume of the *Mercenaria* heart at an afterload of 2 cmH_2O. The data are from two different preparations, and three experimental runs corrected for ventricular weight. Standard errors are shown on each side of the mean values, and as in Fig. 4, open symbols are for ascending levels of preload.

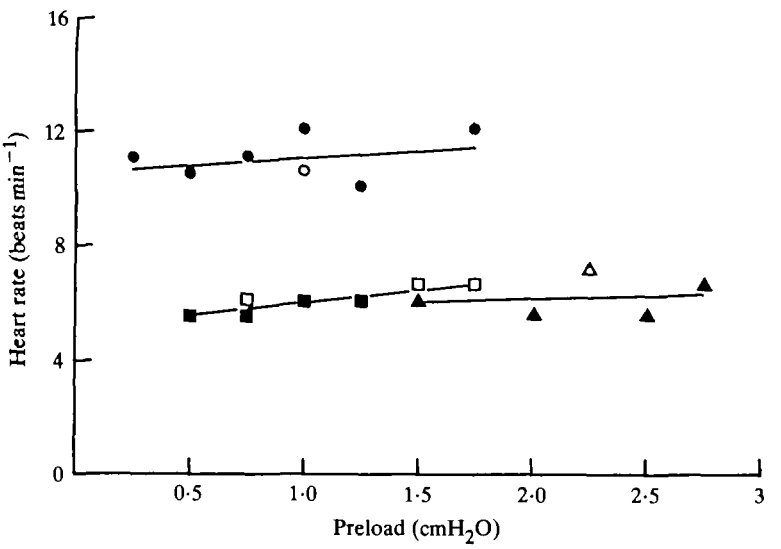

Fig. 10. Contractile rate of the *Mercenaria* heart from three preparations in response to changes in the preload level at preset afterload of either 2 (●, ■) or 3 cmH_2O (▲). Heart rate and preload are not related.

Energetics of the Mercenaria *heart*

As with the heart of *Busycon*, the isolated *Mercenaria* heart showed no sign of coordinated activity without internal perfusion. When perfused at low preload pressures the heart showed regular activity (0·25–1·75 cmH_2O). Despite a regular

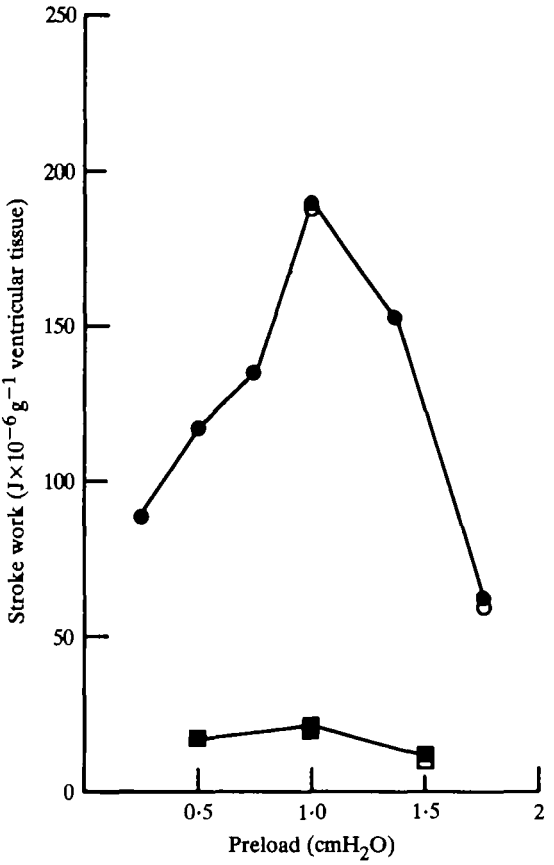

Fig. 11. Stroke work curve calculated from the pooled *Mercenaria* data after correction for ventricular weight. The preload is varied at a preset afterload of 2 cmH_2O. The work curve for *Busycon* at the same perfusion conditions is also shown (■).

contraction, the hearts of *Mercenaria* did not pump the perfusate in three out of five preparations. The reason for this is unclear, but the most likely explanation would seem to be blockage of the posterior aorta or bulbus by the perfusion cannula. The following results are, therefore, based on a very limited sample but they do show some interesting comparative results with the gastropod.

As with *Busycon*, the stroke volume varied in direct proportion to the preload (Fig. 9: $r = 0{\cdot}76$; $P < 0{\cdot}001$), but as the hearts tested would only operate at an afterload of 2 cmH_2O it is not possible to make any statement on the afterload effect. Without more data the failure of the *Mercenaria* heart to pump at higher afterloads may not be meaningful. The relationship between preload and stroke volume was linear with a regression equation of:

$$\text{stroke volume (ml)} = 15 \times 10^{-3}\ P_I + 25 \times 10^{-2}.$$

Unlike in the gastropod heart, the rate of contraction was unaffected over the pressure ranges examined (Fig. 10).

The actual values for the stroke volumes of the *Mercenaria* heart ranged between 161 and 222 μl at a preload of 1·25 cmH_2O with an afterload of 2 cmH_2O. When

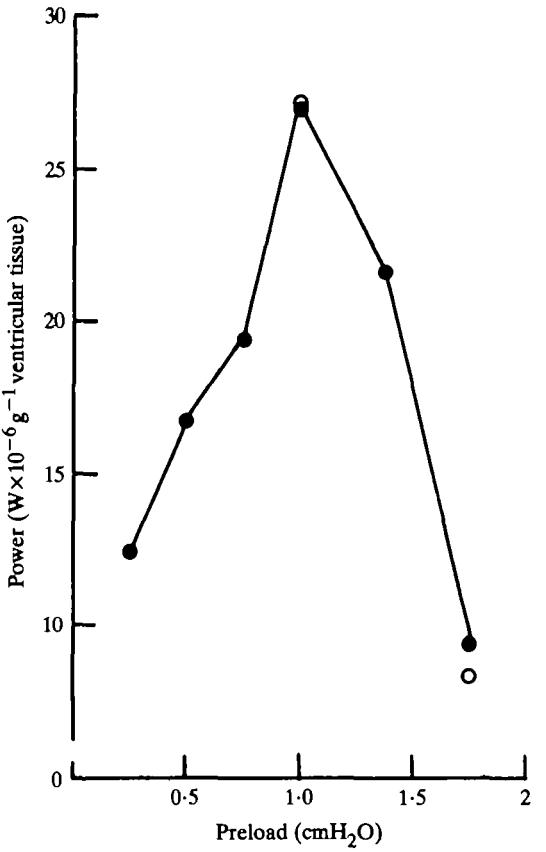

Fig. 12. Power curve for *Mercenaria* calculated from pooling both stroke volume and heart rate data. The afterload is preset at 2 cmH_2O.

corrected for the heart weight the stroke volume per gram is between 1 and 3 ml, over the pressure range examined.

The stroke work and power curves for *Mercenaria* are shown in Figs 11 and 12. As heart rate was very variable between the preparations, the power levels particularly must be treated with caution. What is clear from these data is that the stroke work levels for *Busycon* and the bivalve are very different at the low preload values (Fig. 11). The *Mercenaria* heart is clearly capable of generating a higher work level at preload values of between 0·25 and 1·75 cmH_2O (in both cases the afterload is 2 cmH_2O).

Electrical activity and perfusion pressures

During some of the above experiments a suction electrode was applied to the epimyocardium. This technique records a myogram which reflects the shape of the muscle action potential. The suction electrode technique has several disadvantages but permits the continuous recording of the electrical activity of the functioning heart. The disadvantage results from the thinness of the epimyocardium of both the *Busycon* and *Mercenaria* ventricle. The suction required to hold the electrode on the heart's surface tends to draw perfusate across the wall, inevitably lessening the degree of

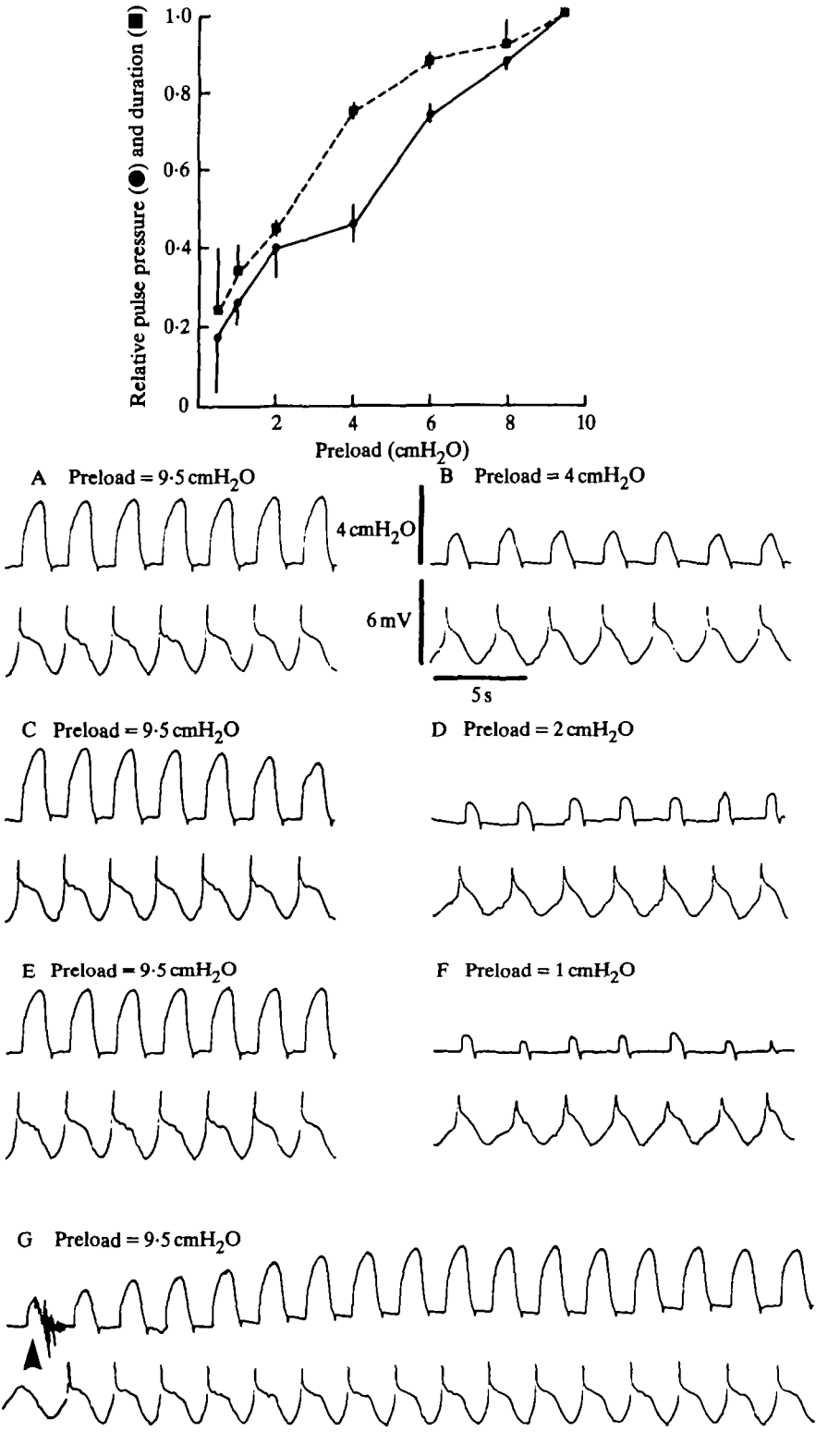
Relative pulse pressure (●) and duration (■)
1·0
0·8
0·6
0·4
0·2
0
2
4
6
8
10
Preload (cmH2O)
A Preload = 9·5 cmH2O
B Preload = 4 cmH2O
4 cmH2O
6 mV
5 s
C Preload = 9·5 cmH2O
D Preload = 2 cmH2O
E Preload = 9·5 cmH2O
F Preload = 1 cmH2O
G Preload = 9·5 cmH2O

Fig. 13

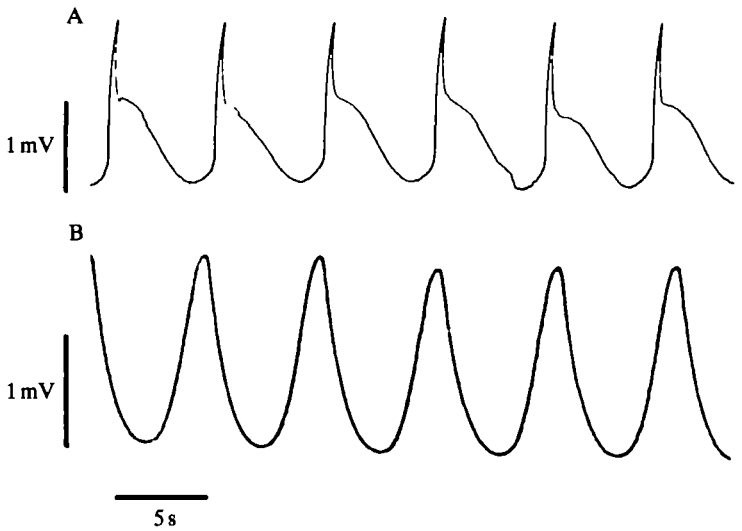

Fig. 14. (A) Suction electrode recording from the surface of the *Mercenaria* ventricle. As in *Busycon*, it has both a rapid spike and slower plateau phase. (B) Sucrose gap recording of the *Mercenaria* cardiac action potential. Note that here the wave form is a slow sinusoidal (reproduced with permission from C. L. Devlin and recorded using the technique described by Hill, 1974).

suction. As the amplitude of the record is dependent on the suction level the records are invariably unstable to a greater or lesser degree. However, provided a predetermined level of perfusion is returned to after each change in preload, a qualitative analysis is possible, although the instability restricts the extent of any experiment.

Several of the *Busycon* preparations show that the form of the myogram is related to the preload value. An individual example, along with the amplitudes and durations of the aortic pressure pulse expressed relative to the performance at a preload level of 9·5 cmH_2O (from four preparations), is shown in Fig. 13. By comparing different preparations two general conclusions can be arrived at and are illustrated by Fig. 13; the first and most obvious is that the prepotential rise time is slower as the preload is decreased; secondly the area under the plateau appears to decline, the time to half-repolarization being reduced as preload gets smaller.

In the heart of *Mercenaria* it was not possible to observe the effects of preload on myogram shape as in this case both the preparation and the suction were unstable. It is, however, very obvious that, as with *Busycon*, the record has a clear spike followed by a plateau phase (Fig. 14A).

Fig. 13. The relationship between preload, the aortic pressure pulse amplitude and duration normalized with respect to the initial values at a preload of 9·5 cmH_2O, and the shape of the myogram recorded with the suction electrode for *Busycon canaliculatum*. Before and after each preload change, the preload was returned to a value of 9·5 cmH_2O. Throughout, the afterload was held at 10 cmH_2O. In the last sequence the changeover point from 1 cmH_2O to 9·5 cmH_2O is shown (arrowhead). The changes in the myogram shape shown in these examples were observed in two other preparations although the stability of the recordings were not as good. Standard deviations are shown for the pressure pulse data ($N = 4$).

DISCUSSION

The results of this study show that over the probable physiological pressure ranges the cardiac output of the isolated and denervated molluscan heart is largely modulated by changes in stroke volume. Heart rate changes are slight. A similar conclusion has been presented for the isolated heart of the cephalopod *Eledone cirrhosa* (Smith, 1981*a*) and *Octopus vulgaris* (L. Foti, I. T. Genoino & C. Agnisola, in preparation). These hearts, however, may not be totally denervated (Smith & Boyle, 1983). *In vivo* experiments on exercising *Octopus vulgaris* confirm that changes in cardiac output are accommodated primarily by modulation of stroke volume (for review see Smith, 1985). A similar conclusion has been arrived at for the fishes (for review see Jones & Randall, 1978). Unfortunately the data on modulation of cardiac output *in vivo* are limited for other molluscan groups. The structure of the heart, however, indicates that volume regulation may well be important in the intact gastropod and bivalve.

The ventricle of both the gastropod and bivalve is a complex trabecular structure (see Hill & Welsh, 1966; Brunet & Jullien, 1937). The same applies for the atria, and is particularly noticeable in the perfused *Busycon* heart. It seems likely that, as in the ventricle of fishes, amphibia and some reptiles (Johansen, 1965) or the bulbus arteriosus of the trout (Priede, 1976), this structure is a solution to Laplace's Law allowing considerable distension while minimizing the risk of a 'blowout'. Such a structure may be characteristic of small hearts which accommodate an increased output by stepping up stroke volume rather than heart rate.

The relationship between preload and stroke volume, for both *Busycon* and *Mercenaria*, conforms to Starling's Law (Patterson & Starling, 1914). It would be expected that, as the preload and afterload exceed physiological limits, a maximum value for stroke volume would be reached, decreasing as the myocardium is stretched beyond its limits of tolerance or when the atrial-ventricular valve malfunctions as in the vertebrates. Over the ranges used, afterload has no affect on output. Afterload does inversely affect stroke volume in *Eledone* (Smith, 1981*a*) and heart rate in *Helix* (Schwartzkopff, 1954).

Since Starling's study it has become increasingly evident that his law may only act *in vivo* to balance the right and left ventricular outputs (Hamilton, 1955; Sit & Vatner, 1982). Increased cardiac output in the mammal is achieved by a decrease in the end-systolic volume rather than an increase in the end-diastolic volume (Asmussen & Nielsen, 1955; Mountcastle, 1974). Working with a very limited sample it seems unlikely that this is the case in the molluscs. The cephalopod, *O. vulgaris*, increases its stroke volume by approximately three times during exercise with only a limited increase in heart rate (for review see Wells, 1983; Smith, 1985). Practically, it would seem likely that this does not result solely from a decrease in end-systolic volume but by increased end-diastolic volume. The interesting question on intrinsic control of the molluscan systemic heart would then be, as Patterson & Starling (1914) originally envisaged for the mammalian heart, 'not . . . how the heart drives the blood round, but the mechanism by which the blood is brought rapidly from the peripheral parts of the body'. Perhaps it is more than a coincidence that cephalopod venous vessels (unlike those of the vertebrates) are contractile (Johansen & Martin, 1962) and a large proportion of the central nervous system is dedicated to the control of the

vascular system (Young, 1971). Ventricular output regulated primarily by the end-diastolic stretch and venous return might also explain why the dibranchiate cephalopods retain large capacitance blood sinuses despite the development of peripheral exchange vessels and how the cardiovascular system in octopus appears to operate normally when the nerves to the ventricle are severed (Wells, 1980). Perturbing the ventricular rhythm can, however, be achieved by cutting the nerves to vessels returning the venous blood to the systemic or branchial hearts (Smith, 1981*a*, *b*). From the present study any intrinsically modulated change in molluscan heart rate is limited but there is no information, except for the cephalopods, on the degree to which cardiac output has to change during exercise.

Relating the cardiac performance of *Busycon* to *Mercenaria* is complicated by differences in heart weights and operating pressure ranges. The comparative difference is highlighted, however, by comparing the stroke work and power levels. Clearly, *Mercenaria* cardiac muscle is capable of generating equivalent work and power outputs at lower preload values than that of *Busycon*. Within the expected *in vivo* pressures of venous return (0·2–1·5 cmH_2O: aortic systolic pressures range between 1·5–6 cmH_2O; Brand, 1972; Florey & Cahill, 1977), the *Mercenaria* heart produces a power output of $15–30 \times 10^{-6} W g^{-1}$ ventricular tissue. This compares with an output of $2–5 \times 10^{-6} W g^{-1}$ ventricular tissue for *Busycon* over the same experimental pressure range. However, at the expected *in vivo* values for the gastropod (atrial pressures = 2–4 cmH_2O; aortic diastolic = 6 cmH_2O: Bourne & Redmond, 1977; Jones, 1970), an equivalent power output is attained ($15–30 \times 10^{-6} W g^{-1}$ ventricular tissue). Given the limitations of the technique used in Herold's study (1975) and correcting his data for heart weight, a power output of $103 \times 10^{-6} W g^{-1}$ ventricular tissue for *Helix* results. Power output levels from *in vivo* results for *O. vulgaris* [Wells, 1979: mean aortic pressure is estimated as $\frac{1}{3}$ (systolic pressure + 2 × diastolic)] give a value around $1·2 \times 10^{-3} W g^{-1}$ ventricular tissue. (An estimate of the heart weight is made from Boyle & Knobloch, 1982.) These values compare with $45 \times 10^{-3} W g^{-1}$ heart weight for man in extreme exercise (Weis-Fogh & Alexander, 1977) and $416 \times 10^{-6} W g^{-1}$ ventricular tissue for the perfused heart of the sea raven [teleost: Farrell, MacLeod, Driedzic & Woods, 1983; a pyramidal heart ratio of 0·167 has been assumed (Santer, Walker, Emerson & Witthames, 1983)]. Calculation of power outputs for other molluscan species *in vivo* is not possible from the data in the literature. Most studies have recorded only intraventricular pressures, so that pressure difference across the heart is not known. Stroke volumes for *in vivo* molluscan cardiovascular systems, other than for the cephalopods, are also difficult to determine except by direct measurement. Indirect determination by Fick's principle is confused by the possibility of considerable extravascular oxygen uptake (Booth & Mangum, 1978).

The results of electrical recording from the ventricle of *Busycon canaliculatum* show that increased perfusion preload increases the rate of rise of the prepotential and the duration of the plateau phase. This observation is in agreement with previous work on the effects of stretch on action potentials of gastropod cardiac muscle. Nomura (1963) used intracellular microelectrode recording from single cardiac trabeculae of *Dolabella auricularia* to relate force to duration of the action potential. Stretch increased the rate of the slow diastolic depolarization and prolonged the duration of

the plateau in proportion to the degree of stretch. Force of contraction increased linearly in proportion to plateau duration. Sudden release of stretch led to an abrupt shortening of plateau duration and diminution in force. Nomura's observations were made with the microelectrode method, using opisthobranch myocardium, but similar observations have been made with the suction electrode method using the heart of a large prosobranch gastropod. Hill & Irisawa (1967) used the cannulated ventricle of *Rapana thomasiana* to observe correlated flow, force and action potentials. A sudden drop in perfusion pressure transformed the action potential from a spike-and-plateau form (like the intracellular action potential of *Dolabella auricularia*) to a sine-wave form (like the sucrose gap action potential of *Mercenaria mercenaria*: Devlin, 1985). A drastic diminution in force was correlated with the loss of the plateau. Active force in the ventricle of *Busycon canaliculatum* is also related to filling pressure (Hill, Kettyle & Tuxen, 1969). Taken together with the present study, these findings support control of cardiac output by duration of the action potential.

Microelectrode recording from cells of ventricular trabeculae of *M. mercenaria* reveals, as in the oyster (Irisawa *et al.* 1961), that there are two types of intracellularly recorded cardiac action potentials (Devlin, 1985). Some cells show a spike-and-plateau form which closely resembles the form of intracellularly recorded cardiac action potentials of *Dolabella auricularia* (Nomura, 1963; Kuwasawa & Matsui, 1970), but other cells show a simple sine-wave deflection. The latter form predominates in the sucrose gap method, suggesting that in the compound cardiac action potential of the horizontally-stretched, collapsed ventricle the inactivity of the cells is producing a simple sine-wave deflection. Thus for hearts which show a predominantly sine-wave form of cardiac action potential in the sucrose gap method, the suction electrode is a preferable method of recording from entire beating hearts.

From this study there are clear indications that within the Mollusca the cardiac muscle of different groups is adapted to work at specific operating ranges. The energetics of the *Busycon* heart are predictable, making this an interesting preparation for examining the modulation of output by both intrinsic and extrinsic factors. Previous research has concentrated on heart rate and the 'force' of contraction. Unpublished work (P. J. S. Smith & R. B. Hill, in preparation) shows that this latter quality need not correlate with stroke volume. The diversity in both form and habitat within this phylum also offers the possibility of examining the properties of the myocardium as it becomes more functionally specialized.

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